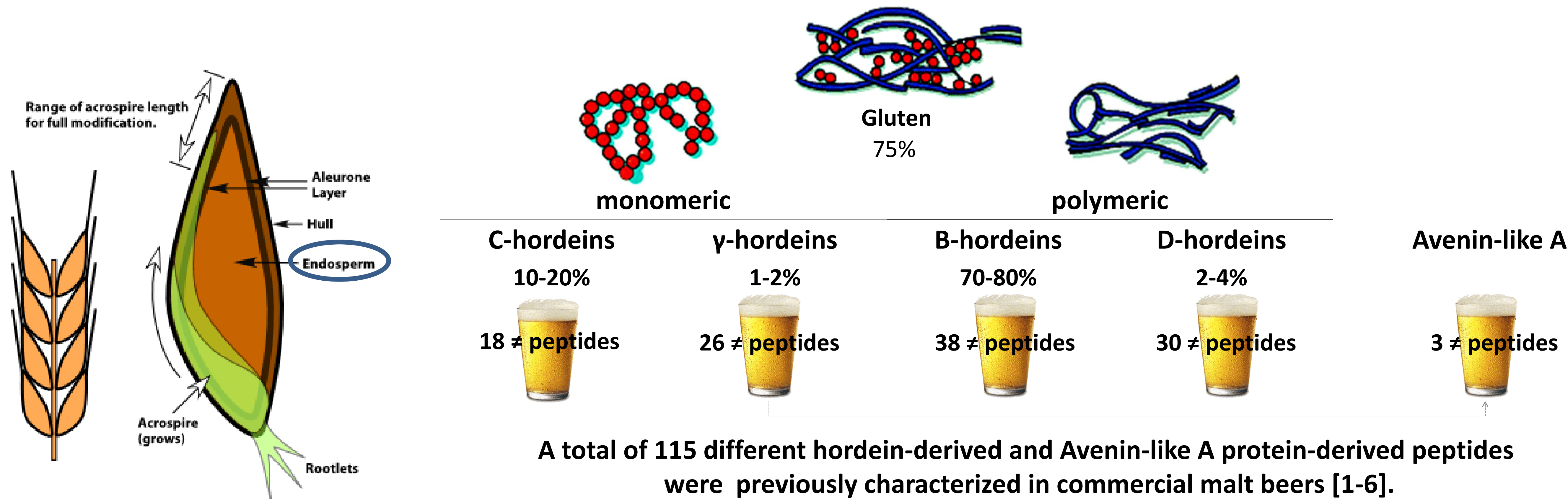


Gluten are one of the 14 official listed allergens by the European Food Safety Authority. Consumption of gluten can cause health implications in individuals with a gluten intolerance or allergy. Awareness of allergens in food and beverages is an upcoming trend in the food industry as well as by regulation authorities. Directive 41/2009/EC defines ‘gluten-free’ (< 20 ppm) food and beverages.

BARLEY HORDEINS AND PEPTIDES IN BEER

Gluten are storage proteins found in the starchy endosperm of barley, wheat and rye kernels. In barley, gluten proteins are named hordeins and account for 75% of the total protein content. Hordeins are a complex polymorphic mixture composed of B-, C-, D- and γ-hordein families. Avenin-like type A storage proteins are foam promoting components that share sequence homology with γ-hordeins.

Malt derived from germinated barley is the basic ingredient in traditional brewing. Through the sequential processes of malting, mashing and brewing hordeins are hydrolysed partially to peptides or completely to amino acids.



IMMUNOGENIC AND TOXIC PROPERTIES OF HORDEIN PEPTIDES IN BEER

Characteristic to all hordeins and derived peptides are the high levels of the amino acid proline making them difficult to degrade by gastrointestinal enzymes. As a result, relatively large hordein-derived peptides enter the small intestine where they can elicit an immune response leading to inflammatory damage in genetically predisposed individuals. So far, 29 immunogenic epitopes causing a T-cell mediated immune response have been described. 39 out of the 115 characterized peptides share a minimal of six residues to one or more of the known T-cell epitopes, rendering them likely to possess immunogenicity.

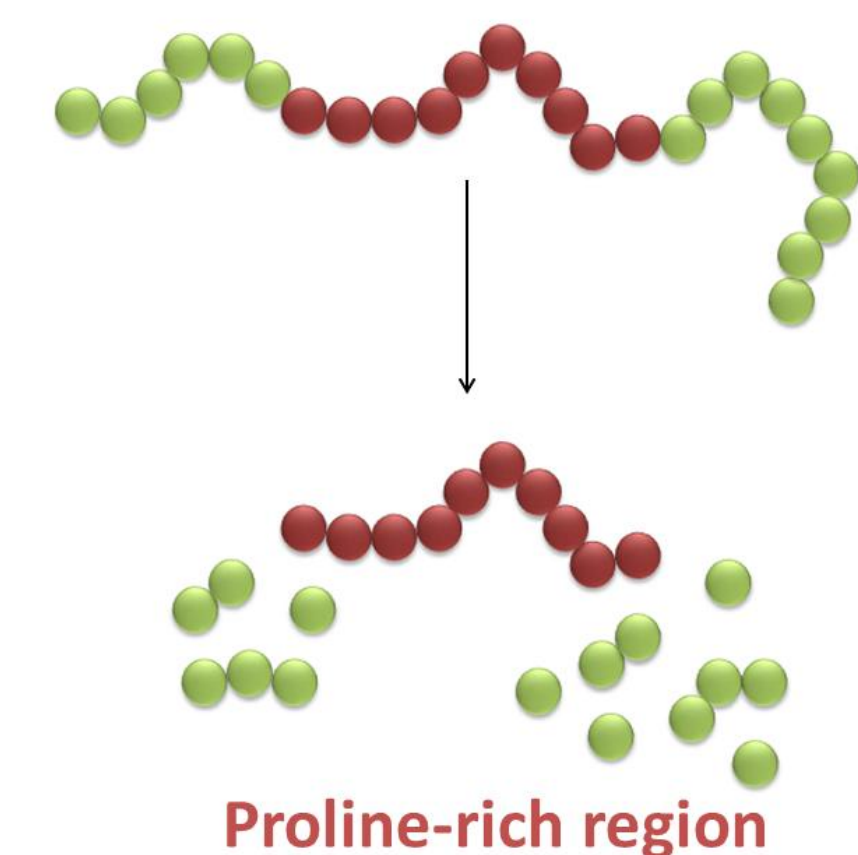


Table: Peptides with a complete T-cell epitope characterized in beers

Hordein	T-cell epitope	Reference	Hordein	T-cell epitope	Reference
	QQPFPQQPQ			QQPQQPFPL	
C	QPQQPFPL <i>QPQQPFPPQ</i>	[3]	C	QQPQQPFPL QPHQP	[4]
C	QIPTPL <i>QPQQPFPPQ</i>	[3]		LIIP QQPQQPFPL QPHQP	[2]
	TPLQP QQPFPQQPQ QPL	[3]		IIP QQPQQPFPL QPHQP	[2]
	QQPQQPFPPQ		AELIIP	QQPQQPFPL	[3]
C	QFPF QQPQQPFPPQ PQ	[3]	ELIIP	QQPQQPFPL QPHQPYTQQT	[3]
C	TPLQPQ <i>QFPFQQPQQ</i> PL	[3]	ELIIP	QQPQQPFPL QPHQPY	[3]
C	IIPQQPFPLQP <i>QFPFQQPQQ</i> PLPQPQQP	[2]	ELIIP	QQPQQPFPL QPHQPYTQ	[3]
	QQPQQPYPQ		ELIIP	QQPQQPFPL Q	[3]
B1	QP <i>QPYPQQPQQ</i> PFPPQ	[5]	C	QIIP QQPQQPFPL QPHQPY	[3]
	PYPQQPQQP		B3	IP QQPQQPFPL QPQQPQFPQQPI	[6]
B1	QPQ PYPQQPQQP FPPQ	[5]		IIP QQPQQPFPL QPQ	[3]

Known T-cell epitopes within hordein-derived peptides are shown in **bold**. Known T-cell epitopes present in opposite direction are shown in *italics*.

DETECTION AND QUANTIFICATION OF HORDEIN PEPTIDES IN BELGIAN MALT BEERS BY HR-ORBITRAP-MS

To comply with ‘gluten-free’ (< 20 ppm) labeling legislation, sensitive and reliable methods for gluten quantification in food and beverages are required.



Standard approach: LC-MS-based methods in MRM-mode

- Relies upon chemical and physical properties of gluten peptides originating from a trypsin digest
 - High degree of specificity
 - A characterization study of gluten peptides in beer after an *in vitro* tryptic digest showed that the majority of the peptides (> 50%) are semitryptic [2]

New approach: **UHPLC-HR-Orbitrap-MS**

- Allows **full-scan MS** experiments with **post-acquisition ion selection**
 - More hordein-derived peptides can be analysed**
 - High mass resolution and accurate mass measurements offers the ability for highly sensitive, accurate and unambiguous detection and quantification of hordein-derived peptides**

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